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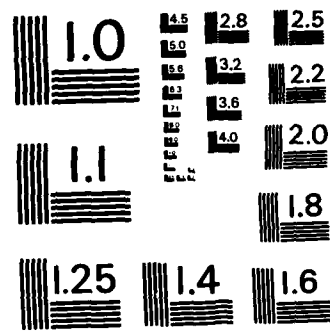
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THE ROLE OF ENDORPHINS IN THE PATHOPHYSIOLOGY
OF HEMORRHAGIC AND ENDOTOXIC SHOCK IN
THE SUBHUMAN PRIMATE

Annual Report

Nelson J. Gurll, M.D.
David G. Reynolds, Ph.D.
Thomas Vargish, M.D.
Carl V. Grisolfi, Ph.D.

September 1, 1982
(September 15, 1981-September 1, 1982)

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The University of Iowa
College of Medicine
Iowa City, Iowa 52242

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20. (continued)

➤ resulted in improved cardiovascular function in both forms of shock and increased survival in hemorrhage. Intact adrenal glands and cardiac autonomic innervation are necessary for naloxone's full effects to obtain, at least in the dog. Central nervous system injection of an endorphin produced bradycardia and hypotension which were reversed by naloxone, but central injection of naloxone did not reverse hemorrhagic shock in the monkey. Opiates failed to release histamine in vivo (rat) and in vitro (rat peritoneal mast cells). The mechanisms of endorphin-mediated cardiovascular dysfunction in shock and its reversal by naloxone are central and not peripheral and involve central nervous system centers controlling autonomic nervous system function or integration.

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SUMMARY

Endorphins acting on opiate receptors are involved in the cardiovascular depression of primate shock since opiate receptor blockade with naloxone and physiological antagonism with thyrotropin-releasing hormone improve cardiovascular function and survival in hemorrhagic and endotoxic shock. The mechanisms and sites of action are central, not peripheral, and involve central nervous system centers controlling the autonomic nervous system.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of The Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. [NIH] 78-23, Revised 1978).

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BODY OF REPORT

A. Problem

Shock due to trauma and hemorrhage is the most serious and lethal threat to the soldier in war. Even during peacetime hemorrhage and septic shock are frequent and important clinical complications in military as well as civilian populations. These shock states do not always respond to seemingly appropriate therapies suggesting the presence of some other pathological condition and, by inference, the possibility of other treatment modalities. Furthermore, the exigencies of the battlefield and rapid evacuation techniques favor the use of specific anti-shock drugs as a temporizing measure until more definitive treatment can be instituted.

B. Background

Endogenous opioid substances are released by stress and, like exogenous opiates, depress cardiovascular function. Endorphins appear to be involved in the cardiovascular pathophysiology of shock as evidenced by improved cardiovascular function and survival using naloxone in canine hemorrhagic^{1,2} and endotoxic³ shock. We have established that naloxone's beneficial effects are dose-dependent,² independent of blood reinfusion,⁴ and reproducible by other opiate receptor antagonists.⁵ Before embarking on clinical studies we decided to investigate the mechanisms of action, doses required, and efficacy in a species closer to man. We also thought this was necessary because of data suggesting that β -endorphin was under a different control mechanism in primates than in other animals.⁶

C. Approach

Cynomolgus monkeys were lightly anesthetized with H_2O/O_2 and instrumented to measure mean arterial pressure (MAP), heart rate (HR), pulmonary arterial pressures including wedge, cardiac output (CO, by thermal dilution), and left ventricular contractility (LV dP/dt max, by differential amplification of LV pressure). Two shock models were used to emphasize the general applicability of these concepts: endotoxemia and hemorrhage. E. coli endotoxin 5 mg/kg (LD_{80}) was given intravenously and the animals treated (when MAP reach 67-75 mmHg or its nadir 15-75 min after endotoxin) with either 0.9% NaCl (as a control) or naloxone 2 mg/kg plus 2 mg/kg·hr intravenously for 3½ hr. Other animals were bled into a reservoir to achieve a MAP of 45 mmHg for 1 hr when the reservoir was clamped (allowing no reuptake of blood) and the animals treated as above; shed blood was reinfused 1 hr after the reservoir was clamped. Survival was followed for 3 days.

To investigate mechanisms we studied anesthetized dogs in shock after various neuroendocrine manipulations had been performed including adrenalectomy, corticosteroid treatment, cervical vagotomy (cardiac parasympathectomy), and stellate ganglionectomy (partial cardiac sympathectomy). Central nervous system involvement was determined by intracerebroventricular injections of opiate agonists and antagonists in unanesthetized non-shocked monkeys and of opiate antagonists in hemorrhaged monkeys. Peripheral mechanisms involving histamine release were evaluated by measuring plasma histamine in shock states and histamine release in-vivo (rats) and in-vitro (rat peritoneal mast cell) after exposure to exogenous and endogenous opiates.

Various neurohumoral interactions were investigated by measuring plasma histamine, β -endorphin, epinephrine, norepinephrine, and dopamine in the above experiments.

D. Results: Naloxone and TRH

We speculated (annual report dated July 1981, for contract No: DAMD 17-80-C-0091) that naloxone's ineffectiveness in primate hemorrhage, in contrast to its beneficial effects in endotoxemia,⁷ was due to attendant acidosis and hypothermia. This was verified this year. When body temperature and acid-base balance were maintained, naloxone increased MAP by 40% and LV dP/dt max by 66% in hemorrhage, a significant difference compared to controls ($p = .006$ by analysis of variance, ANOVA).⁸ Five of 6 naloxone-treated monkeys survived to 24 hr compared to only 2 of 6 controls ($p < .05$ by Fisher's exact test). MAP responses, furthermore, were proportional to body temperature and arterial pH, and plasma β -endorphin levels were proportional to body temperature.⁹

Physiological antagonism of endorphins with thyrotropin-releasing hormone (TRH) increased MAP by 60% and LV dP/dt max by 67% in primate hemorrhage, significantly different from control responses ($p < .001$ by ANOVA).¹⁰ TRH also increased MAP by 50% and LV dP/dt max by 74% in primate endotoxemia ($p < .001$ by ANOVA versus controls).¹¹ Survival was increased by TRH in hemorrhage (4/5 versus 1/5 controls at 48 hrs) but not in endotoxemia.

Cardiac output has been difficult to measure reliably and reproducibly in these small animals.

E. Results: Mechanisms

Investigation of mechanisms in the anesthetized dog revealed that naloxone responses were attenuated (LV dP/dt max) or abolished (MAP and CO) by adrenalectomy and restored by physiological doses of cortisol.¹² Acute bilateral cervical vagotomy and chronic left stellate ganglionectomy each attenuated somewhat the increases in MAP, CO, and LV dP/dt max due to naloxone in hemorrhagic shock.¹³ Vagotomy actually

increased survival so that there were no significant differences between naloxone and saline treatment.

Injectons of D-ala²-met⁵-enkephalinamide into the third cerebral ventricle of three monkeys produced bradycardia and hypotension which were dose-dependent and naloxone reversible.¹⁴ Injection of naloxone into the third ventricle, however, failed to improve MAP in two hemorrhaged monkeys.¹⁵

Opiates failed to release histamine when injected into the intact rat or following incubation with rat peritoneal mast cells, a rich source of histamine. Opiates, on the other hand, did increase plasma levels of epinephrine and norepinephrine 3-5-fold which was naloxone-blockable and -reversible.¹⁶

F. Results: Neuroendocrine substances

Shock increased 4-5-fold the plasma levels of β -endorphin and β -lipotropin (the prohormone for β -endorphin) uninfluenced by type of shock and naloxone treatment (Tables I and II).^{8,17} Histamine, however, was not increased in the plasma with shock (Table III).¹⁸

G. Discussion

The possible role of endorphins and opiate receptors in the pathophysiology of hemorrhagic¹⁹ and endotoxic²⁰ shock now has some firm scientific support. We have generalized these concepts and results to include other models of shock, e.g., splachnic ischemia,²¹ and to other species, especially those closer to man, e.g., the cynomolgus monkey. Naloxone to a great extent has functioned as the tool to pharmacologically probing the role of endorphins and opiate receptor systems in shock. The results with TPH, which has the opposite physiological

effects from endorphins except analgesia, corroborate our hypothesis that the cardiovascular pathophysiology of shock involves endorphins.

The salubrious effects of TRH and naloxone in primate shock give support to their clinical use in shock. Although it took a while to develop these models and to tumble to the importance of temperature and pH, the models are now reproducible and reliable. Temperatures and acid-base balance are important because opiate receptor binding (of naloxone) is inhibited by cold and acidosis²² and endorphin systems are more active with increased temperature.²³

Good therapies are based upon a solid understanding of pathophysiology and basic mechanisms. For this reason we thought that investigation of mechanisms and sites of action for endorphins and naloxone in shock is important. The partial attenuation of naloxone's effects by cardiac sympathectomy or parasympathectomy suggests that autonomic innervation is important in the naloxone response, although perhaps not critically so since naloxone did have some effect. These results are compatible with those which suggest that opiates act centrally to depress sympathetic and enhance parasympathetic output.²⁴ The improved survival with vagotomy in hemorrhage is consistent with vagally-mediated tonically-active negative inotropism.²⁵

The central nervous system control centers regulating and integrating the autonomic nervous system would seem to be the most likely site of action for endorphins mediated cardiovascular depression since central injections of opiates cause hypotension and many of the brain nuclei involved in cardiovascular regulation contain opiate receptors.²⁶ The fact that we were unable to reverse shock by central injection of naloxone is unfortunate but may have been due to adhesions in the aqueductal system of these chronically instrumented monkeys,

preventing access of naloxone to the fourth ventricle where opiates probably have their main effects.²⁷

We expected adrenalectomy to enhance the naloxone effect by loss of corticosteroid negative inhibition of ACTH and β -endorphin secretion. On the contrary and in contrast to our previous results showing that pharmacological doses of corticosteroid attenuate the naloxone response,²⁸ adrenalectomy abolished the naloxone response. This result rules out adrenal enkephalins being responsible for the endorphin-mediated cardiovascular depression. The fact that cortisol restored the naloxone response suggests that the crucial element for the naloxone effect lost by adrenalectomy is the adrenal cortex not, as one might have expected, the adrenal medulla. However, cortisol has a variety of effects (including interactions with receptor membranes, inhibition of catecholamine catabolism, and stimulation of opiate metabolic breakdown) which need to be investigated before one can discern how it restores naloxone's effectiveness.

In contrast to classical teaching, we found that opiates did not release histamine in-vivo and in-vitro. Some of the classical experiments, however, were performed using unphysiological doses of morphine. At any rate, histamine release would seem to play a minor role in the cardiovascular effects of exogenous and endogenous opiates. We did find that morphine increased plasma levels of epinephrine and norepinephrine and that this effect was opiate receptor mediated since it was naloxone-blockable and -reversible. This is compatible with the observation that injection of β -endorphin increases plasma levels of catecholamines²⁹ but seems contrary to the idea that endorphins depress sympathetic function (*vide supra*).

One of the disadvantages of naloxone use in humans is the theoretical possibility of enhancing pain by blocking endorphin-mediated

analgesia. Consequently, TRH is an attractive alternative to naloxone in human shock since it antagonizes all endorphin effects except analgesia. Recently we have come to recognize the existence of multiple opiate receptors each with preferred binding substances and specific effects. The availability of agonists and antagonists for each of the receptor subtypes (μ , κ , δ , σ , etc.) may allow more sensitive pharmacological probing and perhaps the development of more specific agents in the treatment of shock states.

H. Conclusions

Our models of hemorrhagic and endotoxic shock in the monkey are reliable but require attention to detail especially body temperature and acid-base balance. Endorphins are activated in and contribute to the pathophysiology of shock in the primate acting at sites in the brain resulting in adverse autonomic influences on the cardiovascular system. Naloxone reversal of this state requires intact adrenals and cardiac autonomic innervation. Direct central effects predominate over peripheral ones. More specific agents are even more desirable.

I. Recommendations

We should continue to define the precise mechanisms and sites involved. This requires some specific pharmacological manipulations of the autonomic nervous system (α -adrenergic, β -adrenergic, cholinergic-muscarinic antagonists) and even more specific central nervous system injection studies. We should avail ourselves of new opiate receptor subtype antagonists and agonists to probe mechanisms as well as to treat experimental shock paradigms. Our hope is to reverse the cardiovascular depression without affecting analgesia due to endorphins. We need to continue to use monkeys because of their closeness to man but may need

to substitute dogs on occasion in some studies when we would like a more reliable determination of cardiac output (and hence can calculate vascular resistance). Another way around the problem of unreliable low cardiac outputs has been recently described and may prove useful.³⁰

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Table I: Plasma β -endorphin (β -EP, pg/ml) and β -lipotropin (β -LPH, pg/ml) in monkey hemorrhagic shock:

Treat		-30	0	Time from shock, min		120	300
				60	90		
β -EP	Saline	382 \pm 125	1072 \pm 187	1181 \pm 265	1289 \pm 122	1917 \pm 400	583 \pm 228
	Naloxone	230 \pm 64	661 \pm 47	895 \pm 142	988 \pm 164	731 \pm 200	776 \pm 82
β -LPH	Saline	396 \pm 164	1374 \pm 364	674 \pm 168	1936 \pm 498	1189 \pm 632	792 \pm 249
	Naloxone	108 \pm 42	617 \pm 133	798 \pm 246	2316 \pm 1193	370 \pm 57	615 \pm 212

Table II: Plasma β -EP and β -LPH in monkey endotoxemic shock:

Treat		Baseline	Treatment	T+30 min	End
β -EP	Saline	201 \pm 99	521 \pm 118	781 \pm 142	450 \pm 107
	Naloxone	170 \pm 93	605 \pm 118	936 \pm 203	479 \pm 100
β -LPH	Saline	28 \pm 28	373 \pm 166	844 \pm 293	450 \pm 154
	Naloxone	131 \pm 131	685 \pm 292	972 \pm 428	453 \pm 219

Table III: Whole blood histamine (ng/ml) in monkey hemorrhagic shock:

-60	0	Time from shock, min		180	210
		60	120		
13 \pm 2	17 \pm 2	16 \pm 5	16 \pm 3	25 \pm 6	33 \pm 6

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